

AMENDMENTS TO THE SPECIFICATION

Please amend the specification, as follows:

Please replace the paragraph appearing at page 38, immediately following the formulas through the bottom of the page with the following amended paragraph:

The sodium salt (1.0 g) of the galactose-modified CM-dextran polyalcohol obtained in the above (B) was dissolved in water (30 ml), and the solution was added with a solution of trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (150 mg) (~~SEQ ID NO. 1~~) and 1-hydroxybenzotriazole (35 mg) in methanol (40 ml). The solution was adjusted to pH 7.0, and then added with water-soluble carbodiimide hydrochloride (35 mg) 3 times every 2 hours and stirred overnight. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (20 ml), and the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm, 8 minutes). The precipitates were dissolved in water and desalted by ultrafiltration using a Biomax-3 membrane. The residual solution, which did not pass through the membrane, was filtered through a Millipore filter (0.22 μ m), and lyophilized to obtain 900 mg of the title compound. The resulting product was dissolved in 0.1 M aqueous sodium chloride, and analyzed by GPC (column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M aqueous NaCl, flow rate; 0.8 ml/min). The results of the GPC analysis and an ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the compound are shown in Figs. ~~3 and 4~~ 4 and 3, respectively. The DX-8951 content in the compound

was found as 4.9% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

Please replace the paragraph at page 39 immediately following the formulas through the first line of page 40 with the following amended paragraph:

The sodium salt of the CM-dextran polyalcohol obtained in the above (B) (2.0 g) was dissolved in water, and the solution was passed through Dowex-50 WX8 (Et_3NH^+) to obtain triethylammonium salt of CM-dextran polyalcohol (1.9 g). The resulting triethylammonium salt of CM-dextran polyalcohol (1.9 g) was dissolved in an aqueous solution containing 50% of N,N-dimethylformamide. The solution was successively added with a solution of triethylamine (0.112 ml) and trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (350 mg) in N,N-dimethylformamide (10 ml), and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroxyquinoline (1.9 g), and the mixture was allowed to react overnight at room temperature with stirring. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (20 ml), and the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm). These precipitates were dissolved in water, and desalted by ultrafiltration using a Biomax-3 membrane. The residual solution that did not pass through the membrane was filtered by a Millipore filter (0.22 μm), and lyophilized to obtain 1.4 g of the title compound. The resulting product was dissolved in 0.1 M aqueous sodium chloride, and analyzed by GPC

(column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M aqueous NaCl, flow rate; 0.8 ml/min). The result of the GPC analysis and ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the compound are shown in Figs. ~~6 and 9~~ 9 and 6, respectively. The DX-8951 content in the compound was found as 5.2% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

Please replace the paragraph appearing at page 41, line 20 through page 42, line 9, with the following amended paragraph:

The resulting polyalcohol (5 g) was added to an aqueous solution obtained by dissolving sodium hydroxide (13.84 g) in water (150 ml), and dissolved in the solution at room temperature. Sodium salt of monochloroacetic acid (61.6 g) was added to the solution under ice cooling and dissolved in the solution, and then the mixture was allowed to react overnight at room temperature. The reaction mixture was adjusted to pH 8.5, and then low molecular weight fractions were removed by ultrafiltration using a Biomax-50 membrane. High molecular weight fractions were lyophilized to obtain sodium salt of CM-dextran polyalcohol (6.2 g). The molecular weight (gel filtration, pullulan standard) of the resulting product was 428K, and the degree of carboxymethylation per saccharide residue was found as 0.9 by alkalimetry. The resulting sodium salt of CM-dextran polyalcohol (500 mg) was dissolved in water (50 ml), and the solution was added with a solution of Compound 2-2 (400 mg) of Example ~~[[1]] 6~~ in methanol (20 ml) and a solution of 1-hydroxybenzotriazole (160 mg) in

methanol (20 ml). The mixture was further added with water-soluble carbodiimide hydrochloride (120 mg) 3 times every 2 hours, and stirred for 6 hours in total. The solvent in the reaction mixture was removed by evaporation, and the resulting oil was dissolved in water and subjected to ultrafiltration using a Biomax-50 membrane to remove low molecular weight fractions. The residual solution was lyophilized to obtain 600 mg of the desired compound. The galactose content of the product was found as 1.7 per 10 saccharide residues determined by the phenol-sulfuric acid method.

Please replace the paragraph appearing at page 42, lines 10-28 with the following amended paragraph:

The resulting sodium salt of galactose-modified CM-dextran polyalcohol (200 mg) was dissolved in water (3 ml), and the solution was added with a solution of trifluoroacetic acid of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (27 mg) in methanol (3 ml) and a solution of 1-hydroxybenzotriazole (7 mg) in methanol (3 ml). The resulting solution was adjusted to pH 7.0, added with water-soluble carbodiimide hydrochloride (7 mg) 3 times every 2 hours, and stirred overnight. The solvent in the reaction mixture was removed by evaporation, and the resulting residue was dissolved in 3 M aqueous sodium chloride (10 ml), and then the solution was added dropwise to ethanol (100 ml). The deposited precipitates were collected by centrifugation (3500 rpm). The precipitates were dissolved in water, and desalted by ultrafiltration using a Biomax-50 membrane. The residual solution that did not pass through the membrane was filtered by a Millipore filter (0.22 μ m), and lyophilized to obtain 180 mg of the title compound. The product was dissolved in 0.1

M aqueous sodium chloride, and analyzed by GPC (column; TOSOH TSK GelPW-4000XL, solvent; 0.1 M NaCl aqueous solution, flow rate; 0.8 ml/min). The result of the GPC analysis and an ultraviolet absorption spectrum (in 0.1 M Tris buffer, pH 9.0) of the product are shown in Figs. ~~7 and 10~~ 10 and 7, respectively. The DX-8951 content in the product was and found as 3.7% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer containing 30% of acetonitrile (pH 10.0).

Please replace the paragraph appearing at page 44, line 15 to page 45, line 12 with the following amended paragraph:

The resulting N-acetylgalactosamine-modified CM-dextran polyalcohol (200 mg) was dissolved in water (10 ml), and the solution was added with a solution of trifluoroacetic acid salt of Gly-Gly-Phe-Gly-DX-8951 (SEQ ID NO. 1) (30 mg) dissolved in methanol (10 ml), and a solution of 1-hydroxybenzotriazole (30 mg) dissolved in methanol (10 ml). The solution was adjusted to pH 7.0, and added with water-soluble carbodiimide hydrochloride (10 mg) 3 times every 2 hours. The mixture was stirred for 2 hours, and adjusted to pH 8.5. Low molecular weight fractions in the reaction mixture was removed by ultrafiltration using a Biomax -50 membrane. The residual solution that did not pass through the membrane was filtered through a Millipore filter (0.22 μ m) and lyophilized to obtain the title compound (203 mg). The resulting product was dissolved in 0.1 M aqueous sodium chloride and then analyzed by GPC (column; TOSOH TSK Gel PW-6000XL, solvent; 0.1 M acetate buffer (pH 5.0) containing 20% of acetonitrile, flow rate; 0.8 ml/min). The result of the GPC analysis and an ultraviolet absorption spectrum of this compound (0.1 M Tris

buffer (pH 10.0):acetonitrile = 7:3, 0.16 mg/ml) are shown in Figs. ~~8 and 11~~ 11 and 8, respectively. The content of drug compound residue in the product was found as 4.6% (w/w) by quantitative analysis based on absorption spectrophotometry at 366 nm in 0.1 M Tris buffer (pH 10.0):acetonitrile = 7:3.

Please replace the paragraph appearing at page 45, lines 13 to 14 with the following amended paragraph:

Example 10: Measurement of DX-8951 content in ~~CM-Dex-PA-Gly-Gly-Phe-Gly-NH-Y'-CH₂-O-CO-DX-8951 (SEQ IS NO. 1)~~ CM-Dex-PA-Gly-Gly-Phe-Gly-PABC-DX-8951 (SEQ ID NO. 1)

Please replace the paragraph appearing at page 45, lines 15 to 29 with the following amended paragraph:

5 µl of a solution of ~~CM-Dex-PA-Gly-Gly-Phe-Gly-NH-Y'-CH₂-O-CO-DX-8951~~ CM-Dex-PA-Gly-Gly-Phe-Gly-PABC-DX-8951 (SEQ ID NO. 1) (~~Y' means p-phenylene group~~ PABC means p-aminobenzyloxycarbonyl group) prepared as 1 mg/ml in distilled water was added with 95 µl of a papain solution prepared as 2 mg/ml in Britton Robinson buffer (pH 6). The reaction mixture was incubated at 40°C for 4 hours, added with 100 µl of 0.5 N HCl solution containing 50% of acetonitrile, and content of the released hydrolysate [DX-8951] was determined by HPLC. For the HPLC analysis, a Symmetry C18 (4.6 x 100 mm; 3.5 µm, Waters Co.) column was used, and elution was performed with a 0.1% trifluoroacetic acid solution supplemented with an organic solvent (methanol:acetonitrile = 1:2) so as to

be a gradient from 20 to 70% for 12 minutes, and the hydrolysate was detected by fluorescent spectroscopy (Ex. 375 nm and Em. 445 nm). As the result, DX-8951 was eluted at about 5.7 minutes. The DX-8951 content in the above DDS compound was calculated as 4.0% by using a calibration curve prepared with DX-8951. On the other hand, the DX-8951 content was calculated as 3.3% based on UV absorption of the aforementioned DDS compound by using a calibration curve prepared with DX-8951.

Please replace the paragraph appearing at page 45, last line to page 46, line 1 with the following amended paragraph:

Example 11: Measurement of DX-8951 content in ~~CM-Dex-PA-Gly-Gly-Gly-Phe-NH-PABC-DX-8951~~ CM-Dex-PA-Gly-Gly-Gly-Phe-PABC-DX-8951 (SEQ ID NO. 8)

Please replace the paragraph appearing at page 46, lines 2 to 16 with the following amended paragraph:

5 μ l of a solution of ~~CM-Dex-PA-Gly-Gly-Gly-Phe-NH-PABC-DX-8951~~ CM-Dex-PA-Gly-Gly-Gly-Phe-PABC-DX-8951 (SEQ ID NO. 8) prepared as 1 mg/ml in distilled water was added with 95 μ l of a solution of α -chymotrypsin prepared as 2 mg/ml in Britton Robinson buffer (pH 6). The reaction mixture was incubated at 40°C for 4 hours and then added with 100 μ l of 0.5 N HCl solution containing 50% of acetonitrile, and the content of the released hydrolysate [DX-8951] was determined by HPLC. For the HPLC analysis, a Symmetry C18 (4.6 x 100 mm; 3.5 μ m, Waters Co.) column was used, and elution was performed with a 0.1% trifluoroacetic acid solution supplemented with an organic solvent

(methanol:acetonitrile = 1:2) so as to be a gradient from 20 to 70% for 12 minutes, and the hydrolysate was detected by fluorescent spectroscopy (Ex. 375 nm and Em. 445 nm). As a result, DX-8951 was eluted at about 5.7 minutes. The DX-8951 content in the above DDS compound was calculated as 2.5% by using a calibration curve prepared with DX-8951. On the other hand, the DX-8951 content was calculated as 1.7% based on UV absorption of the aforementioned DDS compound by using a calibration curve prepared with DX-8951.